EGCG: RECENT FORMULATION DEVELOPMENTS, CHALLENGES AND OPPORTUNITIES

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Abstract: Epigallocatechin gallate (EGCG) is an herbal source of medicine for treating cancers, with minimal side effects, moreover the outcomes of the drug is directly affected by the various physicochemical characteristic. This parameter attracted the drug discovery scientist to find the way to enhance the effects. The review covers all important finding related to the therapeutics effects of the EGCG, the focus of the review was to highlighted the importance of the advanced drug delivery system which are recent advancement in delivery system such as nanotechnology, and liposomes, which enhances the therapeutic effects and chemo preventive actions of EGCH, The nanotechnology gives various advantages, such as low skin irritation and increased protection of encapsulated drug for topical applications in non melanoma skin cancer enhances drug penetration through skin. In the present data collection, some of the important clinical data is incorporated to analyze the actual drug effect in the human population. The review is the combination of the recent data related EGCG.

INTRODUCTION:

Plants, microbe's, animals and marine life all comes under natural sources, out of which plants are the most utilized natural resource used for applications in the pharmaceutical science.[1]The accessibility and abundance of the compounds still comprises of the major natural source for new drugs.[2] EGCG is the biologically active catechin found in green tea and studied as one of the most chemopreventive compound. epigallocatechin gallate (EGCG) can be synthesized from fully green and facile redox chemistry involving reduction of colloidal iron hydroxide ($Fe(OH)_3$) through green tea (GT) polyphenols produced water-soluble Fe_3O_4 nanocrystals coated with GT extracts. [3]Epigallocatechin-3-gallate (EGCG)is a major polyphenol of Green tea[4].

Epigallocatechin gallate can also be define as the one of the most popular green tea extract produced from its leaves Camellia sinensis, which is having great potential and a healthy beverage consumed worldwide.[5] Epigallocatechin gallate (EGCG) is a potent polyphenolic phytochemical antioxidant green tea extract, use in the topical formulations for prevention of skin cancer.[6] It offers various advantages over conventional therapies because of its widely availability and cheap method to isolate from green tea.[7] Other advantages are it has health-promoting effects such as antioxidation, anti-inflammation and anti-cancer activities can be orally administered and acceptable safety profile interest.[8] Used widely clinically and pharmaceuticals because of its short half-life, low stability and low bioavailability and enormous potential as an anti-cancer agent. EGCG as Apoptosis promoter, used in the recent advances as the cellular and molecular levels of carcinogenesis which led to the development of promising strategies for the chemoprevention of cancers and acts as a natural compounds

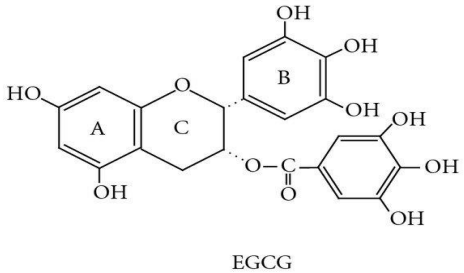
active against skin cancer (Dietary components, phytochemicals and crude extracts).[9] There are various advantages of EGCG used as a cancer preventive agent that are its safety, low cost and bioavailability ,Glycerin-based vehicles are suitable for stabilizing EGCG, minimized immunogenicity and side-effects.[10]

Epigallocatechin gallate considered as potent phytochemical majorly used for the treatment of skin cancer. i.e., basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), which are type of nonmelanoma skin cancers.[11] Basal cell carcinoma is the most common type of non-melanoma skin cancer, which constitutes about 75% of the cases. It is steadily increased and making it a major challenge in terms of management, cures and cost of public health.[12] There are various new formulation techniques comes in research for the treatment of increased nonmelanoma skin cancers.[13] Topical administration of anticancer drugs is one of the interesting alternatives used to minimize side effects and increased its drug targeting and therapeutic benefits. [14]The major challenge of this kind of treatment is to increase penetration of the drug to the skin sufficient levels to kill cancerous cells.[15] To overcome skin barriers and to reach skin malignancies, number of techniques and formulations has been developed for successful favoring penetration of drug into the deep layers of the epidermis.[16] Types of formulation that enhanced/focused the targeting of drug delivery are liposomes, lipid nanoparticles, solid lipid Nanoparticle,gold nanoparticle, polylactic acid–polyethylene glycol nanoparticles, Nanospheres, Nanoemulsion and Phytosomes.[17] Physical method used to improve the drug penetration by topical means for anticancer effects are iontophoresis and electroporation. Herbal phytoconstituents attain greater therapeutic efficacy by using emerging drug delivery systems i.e. [18-19]Nanoparticle which has applied to the vast majority EGCG works on decreasing the probability of cancer cell implantation rather than direct cancer cell cytotoxicity and also lowers the proteolytic activity.[20]

EPIGALLOCATECHIN GALLATE (EGCG):

Source and Chemical Structure:

Epigallocatechin gallate, EGCG is a plant-based compound, originating from tea called catechin. Epigallocatechin gallate, which is a catechins content found higher in green tea compared to others and is further categorized into a larger group of plant compounds known as polyphenolic hydroxyl group.[21] According to various reported studies epigallocatechin gallate found to be a beneficial antioxidative, anti-inflammatory and anticarcinogenic compound. EGCG is, with solubility in water (20°C) of 40 g/l, a soluble powder and sparingly good soluble powder in polar protic organic solvents like ethanol[22-23]Epigallocatechin gallate is an organic compounds of polypropanoids, belongs to a class of flavanoids, which is a small molecule having chemical formula $C_{12}H_{18}O_{11}$ and monoisotropic average weight 458.3717. [24] Other properties are melting point 140-142°C.Its pharmacology acts as to inhibit cellular oxidation and prevents free radical damage to cells. Act as a potential cancer chemopreventive agent. [25]



EGCG

Fig 1: Structure of Epigallocatechin gallate

The main groups of flavonoids include flavonolsflavan-3-ols (catechins), mainly found in tea and their chemical structures are characterized by two benzene rings which are connected by a linear carbon chain and an aromatic chromophor. Camellia sinensis is rich source of Epigallocatechin-gallate, which is stable and water-soluble member of flavanoid group. EGCG is used in the treatment of cancer, because of its inducing apoptosis and cell cycle arrest property in melanoma cells (A374 and Hs-294T), either alone or in combination with other drugs.[25] EGCG also showed pro-apoptotic activity, which is only selective towards melanoma cells and not towards the normal melanocytes. Down regulation of apoptosis inhibits proteins by which EGCG exerts these effects as mechanism, or cell survival promoting proteins (Bcl-2, D1 and cyclin dependent kinase 2 (cdk2), the up regulation of Bcl-2 associated X protein (Bax), a pro-apoptosis protein, the activation of caspases-3, -7 and -9, and by the induction of tumor.[26] As per reported in vitro and in vivo studied on mice model treatment with a combination of EGCG and interferon, it was found that the synergistic anti-proliferative effects against human melanoma cells, which compelling the evidence related to the induction of DNA damage and high genetic mutation frequency in normal lung and skin cells by high concentrations of EGCG, which could possibly cause cancer.[27]

Mechanism of Action:

EGCG plays an important role in lipid metabolism, where it directly interacts with plasma membrane proteins and phospholipids in whole body physiology as well as at the cellular level resulting stimulation of intracellular signaling pathways.[28] Epigallocatechin gallate mediates biological actions by entering to the intracellular compartments, cytosol, mitochondria, lysosome, and nucleus. EGCG's mechanisms of action involves Apoptotic pathways such as proteasome inhibition, which effects dependency on the, cell types, stress condition and concentration of EGCG.[29-30] This inhibitory action mechanism of EGCG can be generalized to other membrane receptor proteins as well. [31] Epigallocatechin gallate has been reported to inhibit the growth factor receptor phosphorylation, such as platelet-derived growth factor receptor,epidermal growth factor receptor, also responsible for the G0-G1 phase arrest, inhibit DNA methyltransferase activity and inhibit aberrant arachidonic acid metabolism.[32] Other are inhibition of mitogen activation protein kinases, the activation of activator protein-1, and cell transformation.[33]

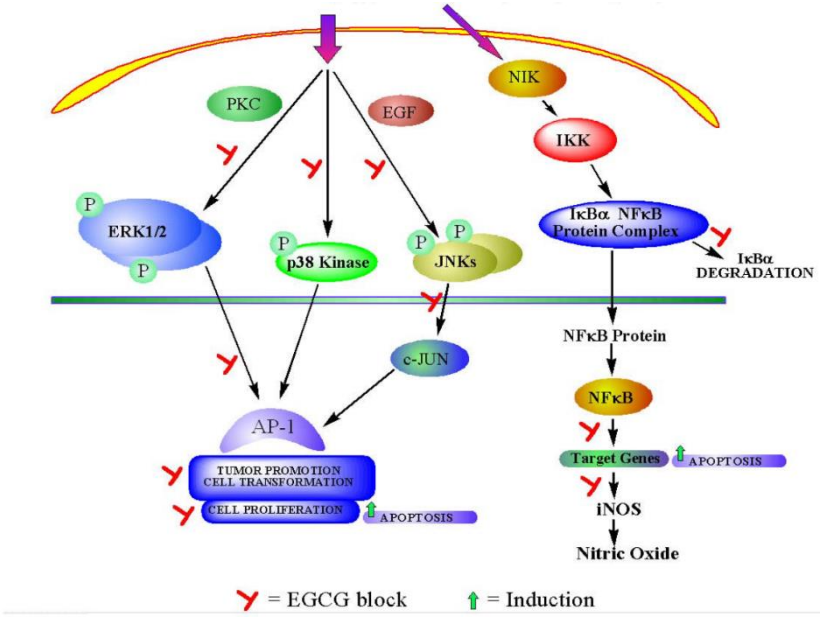


Fig 2: Cancer-related mechanism of EGCG [34]

General Mechanism of action of EGCG for non melanoma skin cancer can be explain as an antioxidative, photoprotectant,inhibition of angiogenesis and induction of apoptosis is mainly promoted by polyphenols which regulate Bcl-2 family proteins result in activation of capase-3, while the down regulation of MMPs expression gives anti migratory effects.[35-36]

Formulation and Challenges:

EGCG is a multipotent anticancer agent, works as a promising molecule in the prevention and treatment of cancer, also offers new clues for discovering multiple-targeted anticancer drugs.[37]Formulation strategies for determining the development of antineoplastic natural compounds highlights the main challenges in the development of optimized drug delivery in anticancer field including all type of skin cancers. Factors limits their bioavailability are physicochemical properties of compounds and their solubility in water.[38]

Ultra violet radiation (B) ranges from 290–320 nm, are mutagenic and carcinogenic in nature and responsible for a variety of skin disease.[39] These radiations induce oxidative stress, DNA damage, immunosuppression and skin cancers including the melanoma and nonmelanoma by penetrating inside epidermis of the skin.[40] There are various types of drug delivery system using EGCG as main drug component to treat cancer. Some are:[41]

Liposomes:

Liposomes consist primarily of phospholipid vesicles, which facilitates the encapsulation of both lipophilic and hydrophilic drugs with higher concentration and are biocompatible, colloidal particles and biodegradable as well.[41] Which is most studied nanocarriers for the treatment of cancer. These vesicles are in turn composed of one or several lipid bilayers According to Fang and group liposome with EGCG for intratumoral delivery was most effective route to treat cancer cells by promoting good quantity of drug deposition at targeted site (neoplastic cells) to treat basal cell carcinoma in female mice.[42]

Nano drug delivery:

Nanotechnology is an emerging drug delivery system to reach at the targeted site, which improves the therapeutic effectiveness and safety profile of conventional cancer chemotherapies.[43] Various types of nanoparticles have been developed for oral, IV and topical applications are solid lipid nanoparticles, polymeric nanoparticles,gold nanoparticles, ß-lactoglobulin-based nanocomplexe, gelatin nanoparticle,PEG nanoparticles and nanospheres as well. These nanoparticles are reported to increase EGCG stability.[44]

Table 1: Type of EGCG Formulations [45-48]

EGCG type of Formulation	Components	Methodof application/routeof administration	Remarks
Nanoparticles	Chitosan nanoparticles (EGCG encapsulated in chitosan nanoparticles)	Orally (enhanceoral bioavailability)	Increased internal organ absorption
Nanoparticles	Polymeric nanoparticles (EGCG into poly lactic co- glycolic acid	Orally (intensifiedDNA damage level in dose dependent manner)	Increases EGCG stability and drug release rate
Gold nanoparticles	T-AUNP loaded with Phytochemical EGCG prepared by ultrasonication method	Oral,Intratumoraland interperitonial injection	Shows five folds higher effective to apoptosis compare to non encapsulated EGCG

Liposomes	Catechin and EGCG in soy phospholipid liposomes	Topical and intratumoral	Higher potency and yield achieved by incorporating EGCG in vesicular structure
Phytosomes	Catechin loaded nano phytosomes	Topically and orally (Better bioavailability, entrapment efficiency)	Phospholipids concentration and addition rate impact on particle size and encapsulation efficiency
Protein based nanoparticle	Oligomer of EGCG, encapsulated drug and protein PEG and EGCG form shell of this	Orally (Boosts cancer efficacy when used with protein drugs)	By these two folds drug accumulated in cancer cells
Nanocarriers	β-Lactoglobulin-chlorogenicacid with EGCG	-	Enhanced EGCG stability in physiological environments. Controlled release in simulated gastric and intestinal environments
Nanocarriers	Gelation coated EGCG	Ocular	Inhibition of HUVECs proliferation and migration. Inhibition of vessel formation in a corneal neovascularization
Nanocarriers	Peptidedendrimers (glycine, proline, lysine,andarginine)	Topical	Enhance the ex-vivo permeation of EGCG
Polymeric nanoparticles	PLGA and PEG	Intravenous	Cultured cancerous cell growth inhibition
Liposomes	Egglecithin and cholesterol	Topical	Enhanced anti MRSA activity
Liposomes	Sorbitan monostearateand cholesterol	Oral	Enhances the cellular permeability in caco 2cellmonolayer

Phytosome:
It is a type of vesicular drug delivery system It is reported by the manufacturer to increase the bioavailability of EGCG in humans

Bioavailability and Stability Challenges of EGCG: As Therapeutic Agent:
EGCG as a therapeutic agent develops so many challenges, out of which bioavailability and stability are two main factors. As EGCG belongs to the class 3 compounds according to BCS classification, which is having dose of 50-300 mg a highly soluble but low permeable drug with an apparent permeability coefficient. In organic solvents EGCG degradation is reduced due to the lack of dissociation and proton-transfer possibilities while some Organic solvents

like diethylene glycol monoethyl ether and glycerinare are option to increases EGCG stability. Factors affects bioavailability of EGCG is due to: [49]

- Cellular upatake is very loe low due to its highly aqueous solubility and low hydrophobicityto cross cell membrane
- EGCG instability in alkaline or neutral condition
- metabolic transformations e.g., Methylation, Glucuronidation and Sulfation
- Multidrug ResistanceAssociated Protein causes active efflux effect of many polyphenolic compounds

The extent of bioavailability also depends on the route of administration and oragn target site. [50]

EGCG in Non-Melanoma Skin Cancer:

Approximately 1.5–2.0 m^2 surface area of body comprises of skin; which is the largest part of body. Skin consists of various functions such as to regulate body temperature, protects body against infections and also Skin is a physical barrier between the external environment and internal tissues thus provides a protective covering to crucial interface between inside and outside and also protect internal organs of the body against the detrimental effects of environmental and xenobiotic agents. [51]

Non-melanoma skin cancer also known as Metastatic or advanced skin cancer.Skin cancer is major public health problem and considered equivalent to all other cancer likes other organs combined Skin cancer, Skin cancer is categorize as melanoma and non-melanoma. Major etiologic factor for initiation of skin cancers is the chronic exposure of the skin to solar ultraviolet (UV) radiation. [52] It is important to protect skin from UVA and UVB both radiations for prevention of non melanoma skin cancer. chemopreventive agents are developed to address this type of issues and disease on urgent basis.[53] Use of polyphenols, EGCG, obtained from green tea is one such strategy becoming popular as a means to protect the skin diseases including the risk of skin cancer. These possess for health effects, organs as well as in general health. Nonmelanoma skin cancers further classified as basal cell carcinoma and squamous cell carcinoma, which are common neoplasms worldwide and are the most common cancers in the United State. EGCG is believed to act as a scavenger of reactive oxygen species and may augment the native antioxidant defense mechanisms of the cell.[54]

EGCG induces tumor cell death via several mechanisms i.e caspase-independent apoptosis, lysosomal membrane permeabilization-mediated cell death, caspase-dependent apoptosis and autophagy. It also inhibits migration and penetration of tumour. As Chemopreventive agent dose of EGCG should be high.[55]

According to Mittal A and group it was reported that long term topical application of EGCG results in high protection against phtocarcegenosis and EGCG also enhance the penetration or absorption capacity inside the skin layers when given in higher concentration.[56]

There are various topical therapies for skin cancer treatment, but comes with the major problem of penetration, the drugs penetrate into the skin by three different routes are: [57]

- Stratum corneum between the corneocytes
- Intracellular route i.e cells and the intervening lipids
- Through hair follicles and sweat glands

So, while making topical application penetration and deposition of drug into skin is major factor, to improve it various approaches are being developed which is as follows: [58]

- **Chemical Enhancers:** Chemicals added with formulation. Fatty acids, oleic acid, azone, dimethyl sulfoxide (DMSO) and terpenes, Propylene glycol, ethanol etc.
- **Physical enhancers by means of electric application**: Iontophorosis, Sonophoresis and Electroporation

- **Advance formulations:**Different type of nanoparticles i.e solid lipid,polymeric,gold , protein and Liposomes.

Future Opportunity:

EGCG is an herbal source of medicine for treating cancers, having less side-effects and toxicity compared to other drugs used, but there are various factors which we cannot ignore while using are its poor absorption, high dose needed for better results. So EGCG holds a great promise for future clinical applications. There is need to focus on the chemical stability of EGCG within the dosage form and methods to enhance the bioavailability of EGCG. For non melanoma Skin cancers its major challenge is to penetrate drug by topical means because of hydrophobic nature of stratum corneum, upper layer of the skin, vechiles required for penetration of drug molecules. As the limited preclinical data and clinical data available in the literature to cure skin cancer by botanical means surgical therapy is the preferred mode of treatment for high-risk Non melanin skin cancer. New methods of prevention need to be developed for non melanoma skin cancer, which is a significant human disease.

Conclusions:

End of several steps of cellular growth lesions namely, dysplasia, metaplasia, hyperplasia and neoplasia are cancer. EGCG is promising molecule and the major bioactive phytochemicals isolated from medicinal plants of green tea having many health benefits, anticancer activity is one of the important function of it. Also, EGCG, Inhibits of all the processes of carcinogen i.e initiation, promotion and progression and has ability to bind and modulate the activity of several signaling molecules related to mitosis, survival, and cellular death, moderating the cellular responses present in cancer.

Advanced drug delivery system which are recent advancement in delivery system such as nanotechnology, liposomes are developed to improve the therapeutic effects and chemo preventive actions of EGCH, holding great promise for future clinical applications. Nanotechnology gives various advantages, such as low skin irritation and increased protection of encapsulated drug for topical applications in non melanoma skin cancer enhances drug penetration through skin.

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